Membrane Tether Extraction from Human Umbilical Vein Endothelial Cells and Its Implication in Leukocyte Rolling

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ABSTRACT During the rolling of human neutrophils on the endothelium, tethers (cylindrical membrane tubes) are likely extracted from the neutrophil. Tether extraction reduces the force imposed on the adhesive bond between the neutrophil and endothelium, thereby facilitating the rolling. However, whether tethers can be extracted from the endothelium is still unknown. Here, with the micropipette-aspiration technique, we show that tethers can be extracted from either suspended or attached human umbilical vein endothelial cells. We also show that a linear relationship between the pulling force and tether growth velocity exists and this relationship does not depend on the receptor type (used to impose point forces), tumor necrosis factor-α stimulation, or cell attachment state. With linear regression, we determined that the threshold force was 50 pN and the effective viscosity was 0.50 pN·s/μm. Therefore, tethers might be simultaneously extracted from the neutrophil and endothelial cell during the rolling and, more importantly, the endothelial cell might contribute much more to the total composite tether length than the neutrophil. Compared with tether extraction from the neutrophil alone, simultaneous tether extraction results in a larger increase in the lifetime of the adhesive bond, and thus further stabilizes the rolling of neutrophils under high physiological shear stresses.

INTRODUCTION

As the initial step of the inflammatory response, human neutrophils roll on the endothelium before their firm adhesion and diapedesis. It has been shown that the rolling is a complicated dynamic process mediated cooperatively by the adhesion molecules expressed on the neutrophil and endothelium (Springer, 1990, 1995), the shear stress due to the blood flow (Finger et al., 1996; Lawrence et al., 1997), and the mechanical properties of the neutrophil membrane (Shao et al., 1998; Schmidtke and Diamond, 2000; Park et al., 2002; Yago et al., 2002).

The adhesion molecules that mediate the rolling process are mainly selectins, i.e., P- and E-selectin on the endothelium and L-selectin on the neutrophil (Springer, 1990; Lawrence and Springer, 1991; Moore et al., 1991, 1995). The binding between selectins and their ligands (e.g., P-selectin and P-selectin glycoprotein ligand-1 or PSGL-1) is characterized by their fast on and off rates. The off rate of a P-selectin/PSGL-1 bond will be increased if a pulling force >25 pN is imposed, demonstrating the slip-bond behavior. However, if a pulling force smaller than 25 pN is imposed, this off rate will be decreased, demonstrating the catch-bond behavior (Marshall et al., 2003). During the rolling process, the pulling force imposed on the adhesive bond by the blood flow is also imposed on the neutrophil and endothelial cell membrane. If a pulling force >45 pN is imposed on a neutrophil surface, a tether (a cylindrical membrane tube tens of nanometers in diameter) will be extracted. Under a constant pulling force, the rate of tether elongation (tether growth velocity) would reach a constant velocity almost instantaneously (Shao and Hochmuth, 1996; Shao et al., 1998). For single tethers extracted from erythrocytes, neutrophils, neuronal growth cones, outer hair cells, and liposomes, the following relationship between the pulling force (F) and tether growth velocity (U_t) has been established (Evans and Yeung, 1994; Dai and Sheetz, 1995; Waugh and Bauserman, 1995; Shao and Hochmuth, 1996; Li et al., 2002)

$$F = F_0 + 2\pi\mu_{\text{eff}}U_t, \quad (1)$$

where F_0 is the threshold force that is determined by the membrane tension, membrane bending stiffness, and adhesion energy between the membrane and cytoskeleton, and μ_{eff} is the effective viscosity that is determined by the membrane viscosity, interbilayer slip, and membrane slip over the cytoskeleton (Evans and Yeung, 1994; Hochmuth et al., 1996).

Under physiological conditions, the point force imposed on the neutrophil membrane by the blood flow can be much >45 pN at the initial arrest of the neutrophil on the endothelium (Shao et al., 1998). Therefore, it is likely that tethers are extracted from the neutrophil during its rolling on the endothelium. Tether extraction from the neutrophil has been shown experimentally in both micropipette and flow chamber studies (Shao et al., 1998; Schmidtke and Diamond, 2000; Park et al., 2002; Yago et al., 2002). Because of tether extraction, the force imposed on the adhesive bond between the neutrophil and endothelium is reduced dramatically, thus increasing the bond lifetime, facilitating the formation of successive bonds, and stabilizing the rolling (Schmidtke and Diamond, 2000; Park et al., 2002; Yago et al., 2002). However, during the rolling process, whether tethers are

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extracted from the endothelium is still unknown. Here, using the micropipette-aspiration technique (MAT) (Shao and Hochmuth, 1996; Shao et al., 2004), we show that membrane tethers can also be extracted from either suspended or attached human umbilical vein endothelial cells (HUVECs). This indicates that tethers might be simultaneously extracted from the neutrophil and endothelial cell during the rolling process, i.e., it is likely a tale of two tethers with one tether from each cell. Compared with tether extraction from the neutrophil alone, simultaneous tether extraction would decrease the force on the adhesive bond faster and provide more assistance in stabilizing the rolling.

MATERIALS AND METHODS

Endothelial cell culture and preparation

HUVECs were purchased from Cambrex Biosciences (Walkersville, MD). They were cultured in 60-mm petri dishes with endothelial growth medium (Cambrex Biosciences) and used at passages 3–5. For experiments with suspended HUVECs, the cells were detached with 2 mM EDTA for 15 min when confluent, washed twice with 0.1% bovine serum albumin (BSA), and resuspended in CO_2-independent medium for immediate use (Invitrogen, Carlsbad, CA). For experiments with attached HUVECs, the cells were cultured for 12 h on cell-culture-treated Thermanox plastic coverslips (NUNC, Naperville, IL), which were mounted on the side wall of the experimental chamber with silicone adhesive (Dow Corning, Midland, MI). Then the chamber was removed from the incubator, washed, and refilled with CO_2-independent medium before experiment. For experiments with cytokine-stimulated HUVECs, the cells were treated in culture with 10 ng/ml tumor necrosis factor-α (TNF-α; R&D Systems, Minneapolis, MN) for 4 h (determined to be the optimum stimulation duration from flow cytometry studies) before experiment.

Antibodies

Mouse anti-human monoclonal antibodies were purchased from three sources: anti-CD29, anti-CD29/FITC, anti-CD31/FITC, anti-CD54/FITC, anti-CD62E, and anti-CD62E/FITC were from BD Pharmingen (San Diego, CA); anti-CD31 and anti-CD54 were from R&D Systems; and mouse IgG1/FITC was from Ancell (Bayport, MN).

Receptor expression on unstimulated and TNF-α-stimulated HUVECs

HUVECs were detached when confluent as described previously. The cells were washed twice and resuspended in FACS buffer (2 mM EDTA and 0.1% BSA in PBS (phosphate buffered saline; pH = 7.4)) at a concentration of 1×10^6 cells/ml before incubation for 45 min on ice with anti-CD31/FITC, anti-CD54/FITC, anti-CD29/FITC, anti-CD62E/FITC (5 μg/ml) or mouse IgG1/FITC (5 μg/ml) and then washed twice with FACS buffer before flow cytometry. To determine the change in the expression levels of CD62E and CD54 on HUVECs after cytokine stimulation, the cells were treated with 10 ng/ml of TNF-α in culture for 4 h before detachment. Flow cytometry was performed on a FACS Calibur system (BD Biosciences, San Jose, CA).

Bead and micropipette preparation

Latex beads coated with goat-anti-mouse antibodies (~8 μm in diameter; Sigma, St. Louis, MO) were washed twice in PBS and incubated with mouse anti-human antibodies (anti-CD29, anti-CD62E, anti-CD31, or anti-CD54) for 1 h at 37°C. The beads were washed twice and resuspended in PBS before use. The bead diameter in solution was determined by dividing their optical diameters (measured with bright field microscopy) by a correction factor (Shao and Xu, 2002). Glass micropipettes of desired diameters (~8 μm in diameter) were prepared with a vertical pipette puller and a microforge as described elsewhere (Shao and Hochmuth, 1996). The narrow opening of the micropipette was filled with 1% BSA and the rest of it was backfilled with PBS. The micropipette diameter was determined with differential interference contrast microscopy and divided by a correction factor (Shao and Xu, 2002). In this study, the gap between the bead and micropipette was ~0.2 μm on average.

Tether extraction

The detailed procedure of the tether-extraction experiment was described previously (Shao and Hochmuth, 1996; Shao and Xu, 2002). Briefly, spherical latex beads coated with mouse antibodies against human endothelial cell receptors were used as the force transducer of the MAT. As shown in Fig. 1 *a*, for suspended HUVECs, the force transducer

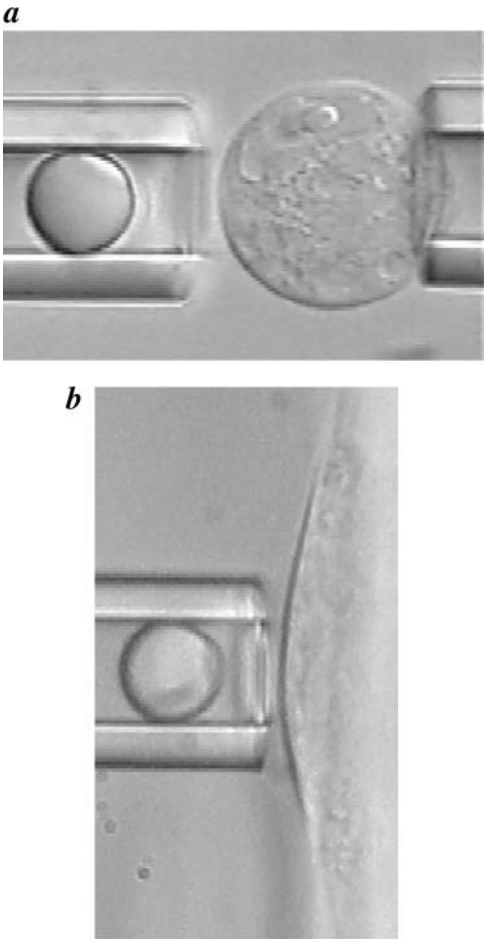

FIGURE 1 The microscopic view of single tether extraction from suspended or attached HUVECs with the micropipette-aspiration technique. (*a*) The experimental setup for extracting single tethers from a suspended HUVEC, which was held with a micropipette as shown. The bead, which fits snugly in the micropipette, acts as the force transducer. (*b*) The experimental setup for extracting single tethers from a surface-attached HUVEC, which was cultured on the side wall of the experimental chamber.

(antibody-coated bead) was aspirated into a micropipette that has a diameter almost identical to the bead diameter. A rounded endothelial cell was held in another micropipette with an aspiration pressure that does not deform the cell too much. For surface-attached HUVECs, a single micropipette that contains the force transducer was used (Fig. 1 *b*). In both cases, a positive pressure was used to drive the bead transducer to contact the cell and an aspiration pressure was used to pull the bead transducer away from the cell. This process was repeated for ~50 times per cell-bead pair at each aspiration pressure (Δp). The whole experiment was recorded on an S-VHS videotape for postanalysis. Typically, the adhesion frequency (the number of adhesion events divided by the number of contacts) was low (<25%, achieved by decreasing the antibody concentration on the bead), indicating dominant single-bond interaction and single-tether extraction between the bead and cell (Piper et al., 1998; Shao and Hochmuth, 1999).

Data analysis

The analysis of the tether-extraction experiment was described in detail elsewhere (Shao and Hochmuth, 1996; Shao and Xu, 2002). Briefly, the S-VHS videotape recorded during the tether-extraction experiment was played in an S-VHS videocassette recorder and the signal was transmitted to a Windows computer through a monochrome frame grabber. The region of interest during one adhesion event was tracked (after being stored as a movie file) with BeadPro8 (a program that has a tracking resolution of ~30 nm and produces the time-displacement data of the bead transducer in an ASCII file) (Shao and Xu, 2002). The tether growth velocity (U_t) and the corresponding free motion velocity of the bead transducer (U_f) were obtained by linear regression. Then the force imposed on the bead transducer (F) can be calculated by Shao and Hochmuth (1996).

$$F = \pi R_p^2 \Delta p \left(1 - \frac{4}{3}\frac{\varepsilon}{R_p}\right)\left(1 - \frac{U_t}{U_f}\right), \quad (2)$$

where Δp is the aspiration pressure inside the left micropipette of radius R_p and ε is the minimum gap width between the bead transducer and pipette wall.

Bond force computation

For simultaneous tether extraction from the neutrophil and endothelial cell during the rolling, the force imposed on the adhesive bond (F_b) and the total tether length (L) were computed by modifying a biomechanical model established earlier (Shao et al., 1998). The equations from geometry, force balance, and torque balance were solved simultaneously with the following equations by numerical means:

$$F_b = 50 + 3\partial L_1/\partial t \,(\text{for the endothelial cell}), \quad (3)$$

$$F_b = 45 + 11\partial L_2/\partial t \,(\text{for the neutrophil}), \quad (4)$$

$$L = L_1 + L_2, \quad (5)$$

and

$$U_t = \partial L/\partial t. \quad (6)$$

In our computation, the radius of the neutrophil was assumed to be 4.25 μm and the shear stress due to the blood flow was assumed to be in the range of 0.09–0.45 pN/μm^2.

RESULTS

Tether extraction from HUVECs

With flow cytometry, we confirmed that both CD29 (β_1-integrins) and CD31 (platelet-endothelial cell adhesion molecule-1 or PECAM-1) are constitutively expressed on HUVECs (Fig. 2, *a* and *b*). Very few nonspecific adhesion events (<0.1%) were observed in our control experiments with uncoated beads or beads coated with anti-L-selectin antibody. No adhesion events were obtained with the addition of function-blocking antibodies against CD31, CD29, CD54, or CD62E to the experimental chamber. The expression of both CD54 (intercellular adhesion molecule-1 or ICAM-1) and CD62E (E-selectin) was upregulated after HUVECs were stimulated with 10 ng/ml TNF-α for 4 h (Fig. 2, *c* and *d*) (McCarron et al., 1995; Panes and Granger, 1998). A significant increase in the adhesion frequency was observed between anti-CD54-coated (or anti-CD62E-coated) beads and TNF-α-stimulated HUVECs as opposed to unstimulated HUVECs.

With the MAT, we extracted tethers from both suspended and attached HUVECs (Fig. 1, *a* and *b*). Membrane tethers usually have diameters of the order of tens of nanometers, so they were barely visible under normal microscopic conditions. To confirm that tethers were indeed membrane tubes, we stained the cell membrane with FM1-43 (Molecular Probes, Eugene, OR) and performed tether-extraction experiments. Fig. 3 *a* shows clearly that the membrane was definitely separated from the cell surface during this process. After the adhesion between the bead and cell was ruptured, the fluorescent membrane tether could be seen retracting back to the cell. Fig. 3 *b* shows the displacement of the bead transducer (D) during one tether-extraction event in a typical experiment. For all the single tether events in our experiments with suspended or attached HUVECs, the time-displacement plots were all similar to this one. If double tethers (two membrane tubes pulled from one cell) were extracted, the bead velocity would increase twice before it reached the free motion velocity (data not shown).

Tether extraction does not depend on receptor type, TNF-α stimulation, or cell attachment state

Shown in Fig. 4, *a–d*, is the correlation between the pulling force and tether growth velocity for single tethers extracted from suspended HUVECs, obtained with beads coated with antibodies to four different surface receptors: CD29, CD31, CD54, or CD62E. Our statistical analysis showed that all the slopes and intercepts do not have any significant difference from each other (for slope comparison, an analysis of covariance was performed; for intercept comparison, the Tukey test was performed; $p > 0.05$ in both cases) (Zar, 1999). These results show that the correlation between F and

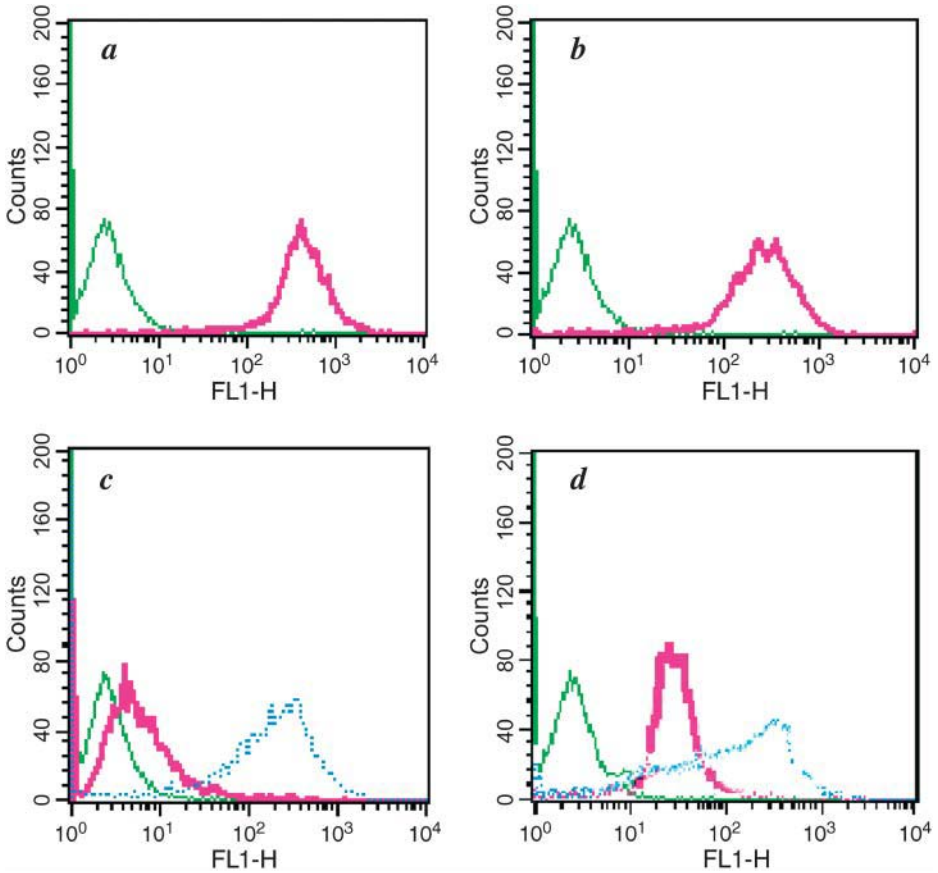

FIGURE 2 Receptor-expression levels on HUVECs. (*a*) Constitutive expression of CD29 (*red*). Green represents the control experiment where HUVECs were labeled with mouse IgG1/FITC. (*b*) Constitutive expression of CD31 (*red*). (*c*) Upregulation in CD54 expression (*blue*) after 4 h TNF-α stimulation (10 ng/ml). Red represents constitutive CD54 expression on unstimulated HUVECs. (*d*) Upregulation in CD62E expression (*blue*) after 4 h TNF-α stimulation (10 ng/ml). Red represents constitutive CD62E expression on unstimulated HUVECs.

U_t does not depend on receptor type or TNF-α stimulation of the endothelial cell.

Shown in Fig. 5, *a–d*, is the correlation between F and U_t for single tethers extracted from attached HUVECs, also obtained with beads coated with antibodies against CD29, CD31, CD54, or CD62E. Statistical comparison among the four regression lines showed no difference in the threshold force and effective viscosity ($p > 0.05$ in both cases). These results strengthen our previous finding that tether extraction is independent of receptor type or TNF-α stimulation. For all the cases, the predicted threshold force and effective viscosity as well as their associated 68% confidence intervals are summarized in Table 1. Multiple comparisons among all the regression lines in Figs. 4 and 5 did not yield any significant difference ($p > 0.05$). Therefore, we can conclude that tether extraction from HUVECs is an intrinsic membrane-protrusion process. For tether extraction from suspended and attached HUVECs, only one constitutive relationship is necessary as shown in Eq. 1 where $F_0 = 50 \pm 2$ pN and $\mu_{eff} = 0.50 \pm 0.03$ pN·s/μm (68% confidence interval). Under physiological conditions, it is likely that HUVECs are morphologically between suspended (rounded) and attached (spread) states; thus we may assume that these parameters are applicable to HUVECs in vivo.

Force decrease due to simultaneous tether extraction

When an adhesive bond is formed between a neutrophil and an endothelial cell in vivo or in a flow chamber, the initial force imposed by the blood flow on this bond (F_b) can be estimated to be ~180 pN at the shear rate of 90 s^{-1} (Shao et al., 1998). This force is large enough to extract tethers from both the neutrophil and endothelial cell. Consequently, two tethers (one from each cell) with the adhesive bond in the middle are likely to be extracted while the neutrophil is rolling on the endothelium (Fig. 6 *a*). By means of a model of mechanical equilibrium (Shao et al., 1998), we can predict how the total composite tether length (L) will grow and how F_b will change over time. As shown in Fig. 6 *b*, at the shear rate of 270 s^{-1}, L will increase rapidly to ~7 μm in 0.1 s. The contribution to L from the endothelial cell (L_1) to the tether length grows much faster than that from the neutrophil (L_2). The relative contribution (L_1/L_2) to the composite tether length will increase further at higher shear rates (data not shown).

Compared with tether extraction from the neutrophil alone (Shao et al., 1998), the initial rate of decrease in F_b is much larger in the case of simultaneous tether extraction (Fig. 6 *c*).

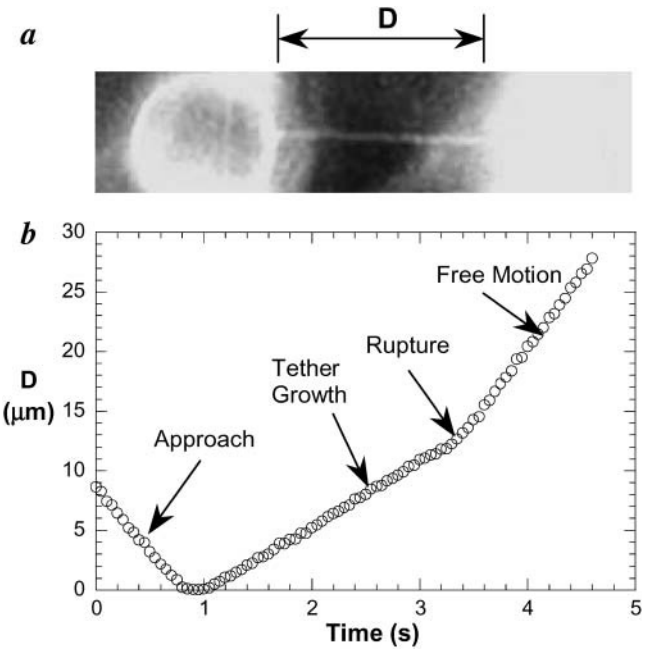

FIGURE 3 Membrane tether extraction. (*a*) Single tether extraction from a suspended HUVEC stained with FM1-43. *D* is the distance between the bead transducer and cell. (*b*) The trajectory of the bead transducer before, during, and after tether extraction in a typical experiment. In this case, a positive pressure was first imposed in the left micropipette to drive the bead toward the cell ($t < 0.8$ s). After a brief contact between the bead and cell (~0.2 s), a negative pressure was imposed on the bead. Because the bead and cell adhered to each other during the contact, so a tether was extracted at a velocity of 5.5 μm/s (1 s $< t <$ 3.3 s). Once the adhesion was ruptured, the bead velocity increased to its free motion velocity of 12.2 μm/s ($t > 3.3$ s). If the bead and cell did not adhere to each other, the bead would move away at its free motion velocity immediately after the contact.

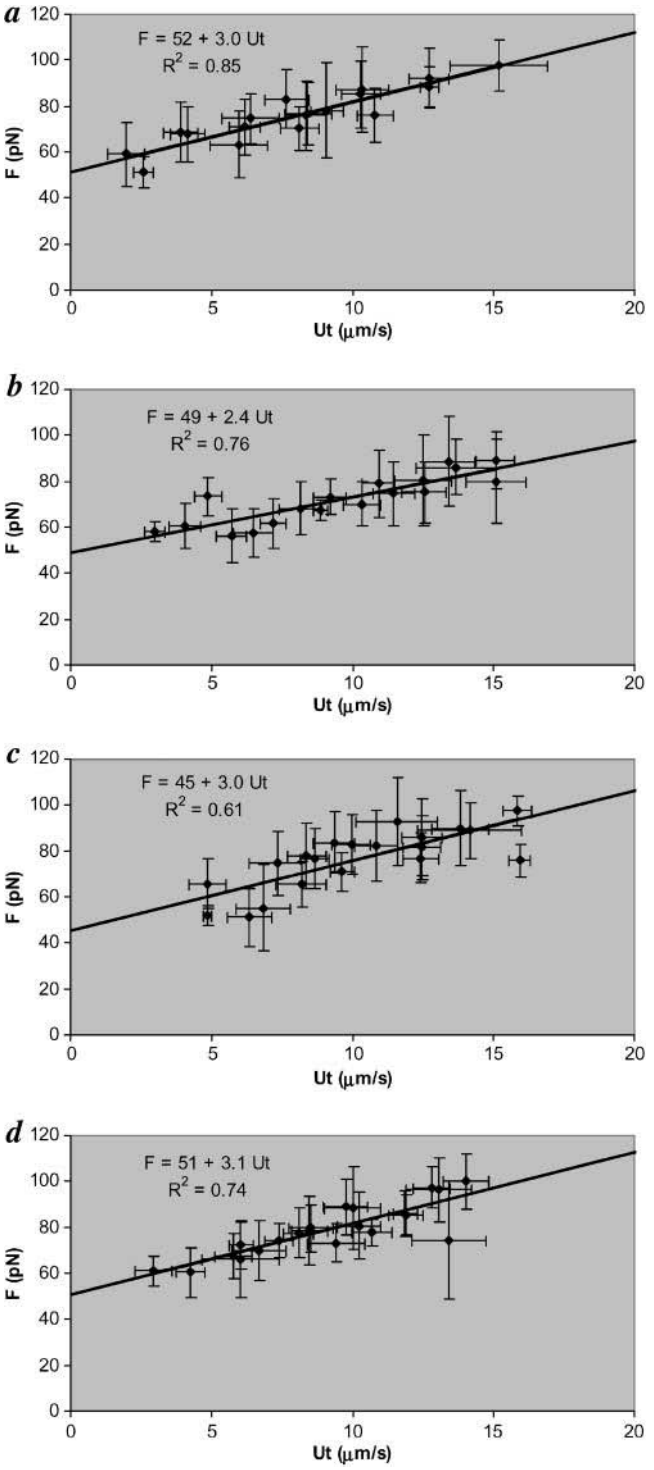

FIGURE 4 The correlation between the pulling force (F) and tether growth velocity (U_t) for single tethers extracted from suspended HUVECs. (*a*) Unstimulated HUVECs with anti-CD29-coated beads as the force transducer, (*b*) unstimulated HUVECs with anti-CD31-coated beads, (*c*) TNF-α-stimulated HUVECs with anti-CD54-coated beads, and (*d*) TNF-α-stimulated HUVECs with anti-CD62E-coated beads. Each point represents an average of 13–25 tethers, obtained with Δp ranging from 0.2 to 0.9 pN/μm^2. All error bars show standard deviations. For each data set, the equations and correlation coefficients obtained with linear regression are also shown.

At higher shear rates, which imply larger forces imposed by the blood flow, this decrease is more prominent due to faster tether growth (Fig. 6 *c*). After 0.1 s, during which the major force decline occurs, F_b would eventually approach $32.05\tau R^2$ (τ is the shear stress and R is the neutrophil radius) or 45 pN (the threshold force for tether extraction from the neutrophil), whichever is larger (Shao et al., 1998). Consequently, at some low shear rates (<86.37 s^{-1}), F_b could become <50 pN (the threshold force for tether extraction from the endothelial cell). For these cases, as soon as F_b falls <50 pN, the tether from the endothelial cell would shrink and L_1 would decrease to zero eventually (data not shown). In any case, it is obvious that in the event of simultaneous tether extraction from the neutrophil and endothelial cell, F_b would decrease more quickly compared with tether extraction from the neutrophil alone, thus increasing the adhesive bond lifetime and making the rolling process more stable.

DISCUSSION

In this study, we extracted single tethers from endothelial cells with beads coated with antibodies against receptors expressed

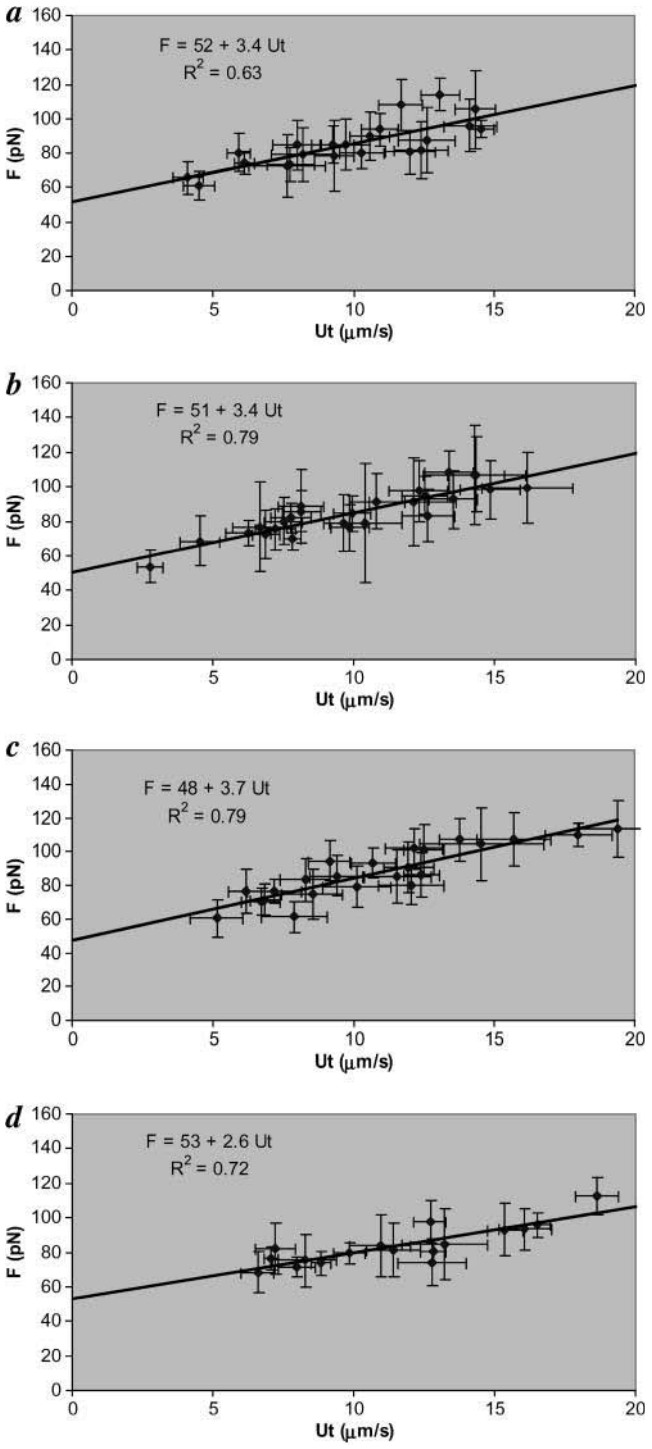

FIGURE 5 The correlation between the pulling force (F) and tether growth velocity (U_t) for single tethers extracted from attached HUVECs. (*a*) Unstimulated HUVECs with anti-CD29-coated beads as the force transducer, (*b*) unstimulated HUVECs with anti-CD31-coated beads, (*c*) TNF-α-stimulated HUVECs with anti-CD54-coated beads, and (*d*) TNF-α-stimulated HUVECs with anti-CD62E-coated beads. Each point represents an average of 14–21 tethers, obtained with Δp ranging from 0.2 to 0.8 pN/μm^2. All error bars show standard deviations. For each data set, the equations and correlation coefficients obtained with linear regression are also shown.

TABLE 1 Predicted threshold force (F_0) and effective viscosity (μ_{eff}), as well as their 68% confidence intervals (Zar, 1999)

Receptor	F_0 (pN)	μ_{eff} (pN·s/μm)
Suspended and unstimulated HUVECs		
CD29	52 ± 3	0.48 ± 0.05
CD31	49 ± 4	0.39 ± 0.06
Suspended and TNF-α-stimulated HUVECs		
CD54	45 ± 6	0.48 ± 0.09
CD62E	51 ± 4	0.49 ± 0.07
Attached and unstimulated HUVECs		
CD29	52 ± 6	0.54 ± 0.09
CD31	51 ± 4	0.55 ± 0.06
Attached and TNF-α-stimulated HUVECs		
CD54	48 ± 5	0.58 ± 0.07
CD62E	53 ± 5	0.42 ± 0.07

constitutively (CD29 and CD31) or upregulated upon TNF-α stimulation (CD54 and CD62E). We determined a linear constitutive relationship between F and U_t for single tether extraction from HUVECs. This linear relationship is remarkably similar for unstimulated and TNF-α-stimulated endothelial cells. Furthermore, when different endothelial receptors were used as a means to impose point forces, this linear relationship changed little. Although suspended endothelial cells are nonphysiologic and their cytoskeleton likely has been reorganized after they are detached, no difference in F_0 and μ_{eff} was found between suspended and attached cells. It should be noted that, compared with cultured cells, HUVECs in vivo likely have their actin stress fibers aligned in the blood flow direction and there is abundant actin near the cell-cell junction. However, it is doubtful that these features have any significant influence on F_0 and μ_{eff} during tether extraction.

F_0 (the threshold force) is mainly determined by the adhesion energy between the membrane and cytoskeleton. For tether extraction from cells with excess area, the adhesion energy can be calculated from F_0. If we assume the bending stiffness of membrane is 0.2 pN·μm (B), the adhesion energy between the membrane and cytoskeleton for HUVECs can be calculated to be $F_0^2/8\pi^2 B \approx 160\,\mathrm{pN}/\mu\mathrm{m}$, which is close to the value for human neutrophils (Hochmuth and Marcus, 2002; Marcus and Hochmuth, 2002).

The effective viscosity (μ_{eff}; slope/2π) is mainly determined by the interbilayer slip and membrane slip over the cytoskeleton. For neutrophils, erythrocytes, and neuronal growth cones, the effective viscosity of tether extraction falls in the range of 0.14–1.8 pN·s/μm (Dai and Sheetz, 1995; Waugh and Bauserman, 1995; Shao and Hochmuth, 1996). The effective viscosity of 0.50 pN·s/μm for HUVECs sits in the middle of this range. The molecular origin of this disparity is still not understood although we know it is likely due to the cytoskeleton, whose composition and organization among different cell types are quite distinct. Smaller effective viscosity implies that less energy is consumed during tether extraction from HUVECs. As a result, in the event of

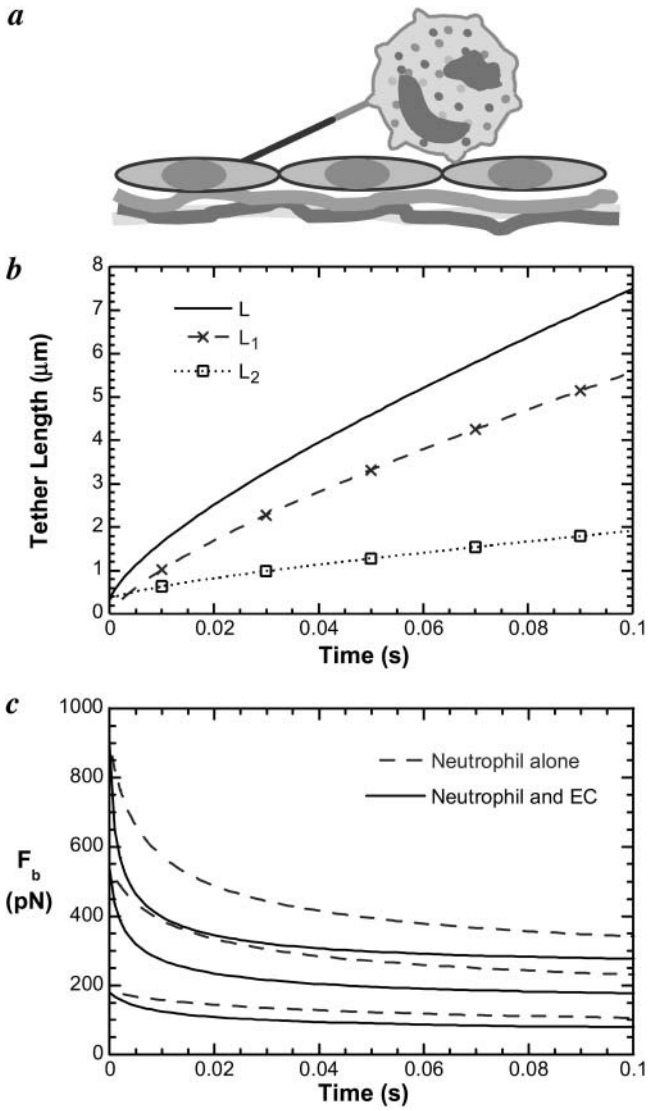

FIGURE 6 Simultaneous tether extraction during the neutrophil rolling on the endothelium and its effect on the force imposed on the adhesive bond (F_b). (*a*) A schematic representation of two tethers extracted simultaneously from a neutrophil (*gray*) and an endothelial cell (*black*). (*b*) The increase in the total tether length (*solid line*) over time determined at the shear rate of 270 s^{-1}. The dashed and dotted lines represent the contributions from the endothelial cell (L_1) and neutrophil (L_2), respectively. An initial microvillus length of 0.35 μm (L_0) was used in the computation. Note $L_2 = L_0$ at time 0. (*c*) The decrease in the bond force (F_b) over time determined at the shear rates of 90, 270, and 450 s^{-1} (from *bottom* to *top*). The solid line represents the case of simultaneous tether extraction, whereas the dashed line represents the case of tether extraction from the neutrophil alone.

simultaneous tether extraction during the rolling process, the tether due to the endothelial cell would be much longer than the one from the neutrophil (Fig. 6 *b*) and this differential in membrane flow would be enhanced with increasing shear rates.

During tether extraction, membrane flows as a consequence of membrane tension gradient. A linear relationship between F and U_t indicates an excess membrane reservoir in both suspended and attached HUVECs, from which membrane materials are drawn to compensate the membrane loss due to tether extraction. If a tether is pulled at a constant velocity without any excess membrane area on the cell, the pulling force would be expected to increase because the membrane expansion modulus is large. Immune cells like neutrophils and lymphocytes store their excess membrane materials in their microvilli, which are membrane protrusions on their surfaces. Suspended HUVECs should have many excess membrane materials after they are detached and become spherical because their ratio of surface area/volume will decrease while their volume likely remains a constant. Attached HUVECs tend to have numerous small membrane blebs on their surfaces (Jaffe et al., 1973). These blebs probably serve as the excess membrane reservoir during tether extraction from attached HUVECs. Because tethers are typically $<$20-μm long and $<$0.1 μm in diameter, not many membrane materials are needed to form a tether.

A neutrophil can roll for ~90 s and 270 μm at a speed of several to 50 μm/s before becoming adherent to the endothelium (Damiano et al., 1996; Schmidtke and Diamond, 2000; Kunkel et al., 2000). The range of tether velocities measured in this study is 2–20 μm/s, which is within the physiological range. It should be clarified that our study deals with the capture and early rolling phase of ''passive'' neutrophil rolling and that it does not consider neutrophil activation associated with later rolling and firm adhesion. Although tether extraction from human neutrophils can be altered by chemoattractants (Shao and Xu, 2002; Marcus and Hochmuth, 2002), tether extraction from HUVECs is independent of TNF-α stimulation. This shows that the effect of TNF-α is fairly specific, i.e., to upregulate the expression of the adhesion molecules (E-selectin and ICAM-1), but not to alter the cell membrane mechanics, i.e., the threshold force and effective viscosity. However, other inflammatory mediators may still synergistically affect the membrane properties of the interacting cells and these potential effects remain to be investigated in the future.

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